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INVESTIGATION INTO THE ADVISABILITY OF SUBSTITUTING AGAR FOR GELATINE AS A MEDIUM FOR THE DETERMINATION OF BACTERIAL COUNTS IN WATER ANALYSIS

BY W. U. C. BATON

In the revision of the standard methods of water analysis, the committee of the American Public Health Association which had this work in charge made a most radical recommendation in regard to bacterial counts. This revision was published in 1912 and the following is quoted from this edition, pages 77 and 78:

In the present state of bacteriology there is no method known by which the absolute number of living bacteria in a sample of water can be determined, and all quantitative determinations of bacteria are necessarily of a relative character. This being the case, strict adherence to a standard procedure is of especial importance. . . .

Quantitative bacterial determinations are of especial value as affording the best index of the efficiency of filtration. . . .

Since gelatine does not give the total number of bacteria in the water, the committee has thought it wise to use Agar incubated at 37° C. as a standard medium. This admits of counts in one day instead of two and give results on the kind of bacteria growing at blood temperature and therefore nearly related to pathogenic types.

The standard medium for determining the number of bacteria in water shall be nutrient agar. All variations from this shall be considered special media.

There can be no doubt as to the stand of the committee. By this report gelatine is absolutely discarded for use in determining the number of bacteria in water. The writer was completely astounded by this extremely radical change. It evidently, judging from adverse expressions which have come to me, affected many others in a similar manner.

A protest has been made against this in a vigorous manner by various men interested. Whipple reports (*American Journal of Public Health* 1913, Vol. 3, No. 1) that a letter of inquiry to twenty leading filter operators brought eighteen replies. A large majority of these either preferred the gelatine or withheld a statement of opinion until they had given the two media a trial side by side.

It is consoling that the laboratory section of the American Public Health Association passed a resolution at the 1912 meeting advising the use of both gelatine and agar and encouraged the gathering of data on which to base a conservative opinion before making so radical a move as proposed by the committee.

As soon as the published report of the committee came to the writer's attention, he at once began to plan the acquiring of data either to justify or disprove the wisdom of the change. He could not conscientiously accept it without a local investigation. The results obtained will prove the wisdom of this apparent distrust and perhaps ultra conservatism.

The writer's previous experiences with agar for the determination of the number of bacteria in water had not been very encouraging. Previous to this time he had always worked with neutral 1.5 per cent agar with or without sugar and litmus. The agar recommended by the committee was different and therein was a possibility that perhaps his adverse opinions were ill conceived.

The work of investigation was outlined as follows:

Four of our routine sampling points were selected for the comparative tests. These represent the waters we have under control. These waters are designated in the tabulations as Class A water, Class B water, Class C water and Class D water.

The frequency of positive tests for coli, also shown in Tables 1 to 4, inclusive, will give an idea of the relative sanitary character of the waters.

The gelatine medium used was made in accordance with standard procedure. The percentage of gelatine was corrected for moisture. The agar medium used was that recommended in the 1912 edition of Standard Methods. This was made up on the basis of dry agar. The gelatine plates were incubated at 20° Centigrade for forty-eight hours. The agar plates were incubated at 37.5° C. for twenty-four hours. Porous covers were used on all agar plates.

The work was begun in October, 1912, and has been continued to the present time. In all some 1898 samples are tabulated. The data submitted extends over a period of sixteen months to February 1, 1914.

Tabulations 1 to 4, inclusive, show monthly averages for the various classes of water previously enumerated. These averages have been further distributed by averaging together only those samples having the same result on the Colon test. In each of these tables, each successive column is indicated as a water of a lower sanitary grade.

TABLE 1
Class "A" water.—Comparison of gelatine and agar counts for various coli conditions

	NEGATIVE 1 CC.			POSITIVE 1 CC.			POSITIVE $\frac{1}{2}$ CC.			POSITIVE $\frac{1}{10}$ CC.		
	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar
1912												
October.....												
November.....												
December.....												
1913												
January.....	5	6,920	405	8	9,088	503	2	31,250	1,125	5	22,200	1,144
February.....	3	8,226	803	7	11,929	666	6	20,267	413	6	60,733	1,093
March.....				2	11,150	248	10	15,190	485	6	10,950	456
April.....				4	98,750	13,275	7	132,286	23,300	8	23,813	1,553
May.....				2	105,000	27,750	9	175,278	55,744	6	184,250	54,000
June.....							8	116,750	30,125	9	51,278	26,700
July.....							1	16,500	6,000	6	27,667	16,600
August.....							4	27,250	21,000	4	18,250	12,025
September.....				2	23,700	4,700	3	23,000	16,067	5	38,800	38,800
October.....	1	350	475	2	17,800	3,800	4	8,850	5,975	3	27,800	9,400
November.....				3	15,100	2,517	6	16,050	675	7	36,927	1,536
December.....	1	4,400	180	7	5,757	187	8	10,113	209	10	4,450	131
1914												
January.....	4	556	13	8	293	260	8	2,581	201	5	4,650	398
										4	6,725	131

TABLE 3
Class C water.—Comparison of gelatine and agar counts for various Coli conditions

	NEGATIVE 10 cc.			POSITIVE 10 cc.			POSITIVE 1 cc.			POSITIVE 1/10 cc.		
	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar
1912												
October.....	3	643	127	11	465	113	7	741	132
November.....	1	4,500	180	13	1,394	59	7	964	58	2	10,250	11
December.....	2	2,388	21	12	1,514	51	5	3,960	80	4	2,125	38
1913												
January.....	7	236	32	8	1,461	91	10	1,335	50	1	1,800	55
February.....	6	5,892	34	16	5,800	41	2	13,500	55
March.....	4	75	19	14	319	28	7	803	23	1	750	17
April.....	10	251	43	11	217	20	2	320	192	1	250	3
May.....	16	447	23	9	436	76
June.....	16	215	17	9	174	24
July.....	3	105	27	19	92	34	3	167	35
August.....	13	28	18	9	42	34	1	25	28
September.....	12	38	55	10	116	78	3	67	56
October.....	4	65	16	11	109	28	11	368	77	1	170	11
November.....	1	900	27	13	1,175	35	8	3,275	28	2	5,350	38
December.....	5	1,452	5	16	970	11	3	1,367	4
1914												
January.....	10	3,275	8	13	1,128	10	2	2,050	2	1	190	3

TABLE 4
Class D water.—Comparison of gelatine and agar counts for various Coli conditions

	NEGATIVE 10 cc.			POSITIVE 10 cc.			POSITIVE 1 cc.			POSITIVE $\frac{1}{10}$ cc.		
	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar
<i>1912</i>												
October.....	17	25	18	6	357	53	1	155	34
November.....	18	153	20	8	2,023	26	1	190	25
December.....	18	60	11	8	1,625	27	4	565	13
<i>1913</i>												
January.....	22	50	16	8	697	42
February.....	20	51	10	3	215	10	1	240	20
March.....	22	22	8	4	135	43
April.....	24	11	8	5	62	22
May.....	27	16	8	2	56	15
June.....	27	12	6	1	45	4
July.....	17	14	9	10	37	14
August.....	24	8	6	3	7	38
September.....	22	13	10	5	67	56
October.....	21	3	4	4	27	46	2	312	14
November.....	19	41	4	3	733	7	2	2,200	10
December.....	28	85	1	2	165	7
<i>1914</i>												
January.....	26	144	2	3	307	9

It is a fact as stated by the committee that there is no method known by which we can get a quantitative estimation of all the living bacteria in a sample. Doubtless a large number of the bacteria which grow at 37.5° C. are closely related to the pathogenic types. That this number is large enough in proportion to cause a definite fluctuation in count directly comparable to the sanitary quality of the water was a question about which the writer was very skeptical. It was this sincere doubt together with lack of definite information on the interpretation of results from agar at 37.5° C. that made him hesitate to discard, point blank, the gelatine method with which he had had years of experience for the agar method with which he had had only unsatisfactory experience and which had apparently so little data behind it.

We knew not what it really meant in terms of gelatine or coli for general analytical work. We were further ignorant of its real value in the estimation of purification efficiency. If you should get 100 per cent removal, what does it mean? It means undoubtedly a zero count on the purified water and that appears good. On the other hand, suppose you have a calculated reduction of 80 per cent; does that mean the purified water was high or the raw water low? It does not necessarily mean low efficiency as efficiency should be reckoned.

All who are familiar with purification works know well that a statement of the percentage removal is about as useless a thing as anyone ever took time to calculate. Actual and comparable counts of the before and after treatment are the only figures upon which we can place any dependence. Even these are very insufficient when it comes to passing final judgment on the value of the water from a sanitary standpoint.

The Colon group of organisms is, at present, the best indication we have of the sanitary condition of the water. Since this is true, the writer took the results of the Colon test as the basic measure in studying the relative value of agar and gelatine counts. All data is presented with this idea in view. It should be stated, however, that the factors affecting bacterial flora in any surface water are many. Temperature, seasonal, climatic, topography, geology and arability of the water shed are among the usually most important. In the case of the western Pennsylvania water, we may add industrial and trades wastes and the acid streams of the mine regions and the result brought about by a conglomerate mixing of these with an uncertain

alkalinity from limestone sections. This brings us to a point where we are ready to discuss the data presented.

A study of Tables 1 to 4, inclusive, brings out no definite relation between the gelatine and coli, the agar and coli or the gelatine and agar. Of course there are certain apparent relations, but they show more exceptions than rules. They vary seasonally and they vary with the classes of water. It is not in the least surprising that this should be so.

In Table 5 the true averages for the entire sixteen months, for the various classes of water, and coli, conditions are given. If any useful relation exists it should be here. In all of these tabulations, the writer believes that the choice is for the gelatine rather than the agar.

Tables 6 and 7 go a step further and give the ratio of agar to gelatine for each class of water and coli condition. In addition the average monthly temperature of the water is given. It is here that there is a marked relation.

In general, the high ratios go with the high temperatures and the low ratios with the low temperatures. This may be partly explained by the fact that in the other tables the agar and gelatine counts fluctuate in opposite direction in respect to temperature.

That the gelatine counts go up as high as shown in cold weather is due in no small way to the existence of low temperature organisms of soil origin. On the other hand, the agar count naturally falls off in the cold weather and goes up in warm weather. It does not follow however that the agar count is higher at the period of highest concentration of pollution, in fact, the data show that it is not.

It is a well known and accepted fact that there are many 37° bacteria in soil that have no sanitary significance. The data here presented bear this out.

Low agar counts on effluents at filter plants in cold weather, would indicate improved working conditions, yet every one knows that such is never the case.

The data presented is perfectly frank in withholding any definite relationship but does show a good seasonal variation. The writer sees no necessity for his further discussion of this data at this time. However, there are certain other points concerning the question of replacement of the gelatine by the agar count which should be taken up.

TABLE 5
Comparison of gelatine and agar counts for various Coli conditions. Counts shown here are the average for sixteen months

B. COLI	CLASS A WATER			CLASS B WATER			CLASS C WATER			CLASS D WATER		
	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar	No. of samples	Gelatine	Agar
Negative in 10 cc.....							113	920	30	352	44	8
Positive in 10 cc.....							194	1,075	43	75	571	29
Negative in 1 cc.....	14	4,741	367	36	9,313	845						
Positive in 1 cc.....	52	46,590	3,386	113	42,982	5,430	71	1,655	56	11	715	16
Positive in 1 $\frac{1}{2}$ cc.....	102	45,511	11,728	166	35,641	6,443	13	3,297	26			
Positive in $\frac{1}{2}$ cc.....	94	37,762	11,177	143	33,106	6,345						
Positive in $\frac{3}{8}$ cc.....	93	35,677	9,129	101	31,727	6,821						
Positive in 1 $\frac{1}{8}$ cc.....	104	49,175	19,393	56	38,632	11,680						
Total samples.....	459			615			391			438		

TABLE 7
Showing ratio of agar to gelatine counts for Class C and Class D waters, with their average monthly temperature, Centigrade

	TEMP. CENT.	NEGATIVE 10 CC.		POSITIVE 10 CC.		POSITIVE 1 CC.		POSITIVE $\frac{1}{10}$ CC.	
		Class C	Class D	Class C	Class D	Class C	Class D	Class C	Class D
1912									
October.....	15	0.197	0.721	0.243	0.148	0.178	0.219
November.....	8	0.040	0.131	0.052	0.013	0.060	0.131	0.001
December.....	3	0.009	0.183	0.034	0.017	0.020	0.023	0.018
1913									
January.....	4	0.135	0.320	0.062	0.060	0.037	0.028
February.....	2	0.006	0.196	0.007	0.046	0.004	0.083
March.....	5	0.253	0.364	0.088	0.318	0.029	0.023
April.....	9	0.171	0.727	0.092	0.355	0.600	0.012
May.....	17	0.051	0.500	0.174	0.268
June.....	21	0.079	0.500	0.138	0.089
July.....	25	0.257	0.642	0.370	0.379	0.209
August.....	25	0.644	0.750	0.810	5.43	1.12
September.....	21	1.44	0.769	0.672	0.837	0.835
October.....	16	0.246	1.33	0.256	1.70	0.209	0.045	0.065
November.....	8	0.030	0.096	0.030	0.010	0.009	0.005	0.007
December.....	4	0.003	0.012	0.011	0.042	0.003
1914									
January.....	2	0.002	0.014	0.009	0.029	0.001	0.016

There is practically no difference in the ease or difficulty with which the two media are made up. The one has no advantage over the other. The agar at 37° C. has an apparent advantage in that it will give a count at the end of twenty-four hours. On the other hand, it has the disadvantage of giving many lost results due to troublesome spreaders even with the porous cover dishes. The writer finds that the loss by liquefying of the gelatine plates is practically nil, hence the results on gelatine, while they take longer, are more certain.

It has been the writer's experience that the gelatine count will frequently indicate conditions which would never be shown by the agar 37° count alone. Such conditions as an abnormal fluctuation in a reservoir, breaks or other abnormal drafts on pipe lines, abnormal growths in filter underdrains, springs and wells sensitive to local rains through shallow drainage and other similar problems which many have doubtless encountered. These may not have an immediate direct bearing on the public health, yet they are conditions which we wish to detect.

We are not getting full efficiency from our bacteriological tests unless we do. The agar 37° count in connection with the gelatine often emphasizes these conditions but many of them it would never detect alone. Furthermore, the agar 37° count on the better class of waters is so low that it would take considerable audacity to say that there was a different interpretation to be placed on a fluctuation of 5 or 10 per cubic centimeter.

One hundred per cubic centimeter or less on gelatine at 20° is the often quoted German standard for good water. This is no doubt reasonably correct, yet we have seen waters with this low count which were still suspicious. On the other hand, we have seen waters with higher counts which we felt were reasonably good.

The agar 37° count which corresponds to this is probably somewhere between 0 and 100, but where? The writer has not the courage to fix the standard from any data in his possession. In fact the more he studies bacteriological data the more convinced is he that the setting of fixed standards is poor policy. Results should be interpreted from knowledge of all related conditions by a man of experience in these matters.

Take Tables 3 and 4, the gelatine shows a markedly wider range of fluctuation and consequently is a much better indicator of working conditions. On the other hand, the agar has no advantage as an indicator of sanitary conditions.

In conclusion, the writer wishes to add his word of protest against the replacement of gelatine 20° C. counts with agar 37° C. counts as standard procedure in water analysis. If it is possible to make only one count the gelatine should be used as it is more reliable and servicable.

If possible, both gelatine counts at 20° C. and agar at 37° C. should be made as it gives to the experienced man more data for his diagnosis and points will often be brought out more completely when we have the two counts.

The value of the two counts over the one will be constantly increasing as we become better acquainted with interpretation of such results.